

Online Figure I. Densitometry analyses of the ratios of phospho-eNOS Ser-635 or Ser-1179 to total eNOS and phospho-AMPK Thr-172 to α -tubulin examined by Western blotting in BAECs treated with various concentrations of AICAR for 15 min (A) or infected with Ad-AMPK-CA at different MOI for 24 h (B). The control cells were infected with Ad-null virus at 50 MOI. *p<0.05 between treated groups and non-treated controls.



Online Figure II. BAECs were treated with H-89 (50 nM) or KN-93 (1 μ M) for 30 min or infected with Ad-null control virus (50 MOI) or Ad-Akt-DN (50 MOI) expressing a dominant mutant of Akt. The cells were then subjected to a laminar shear stress at 12 dyn/cm² for 1, 2, and 5 min. The collected cell lysates were analyzed by Western Blot with various antibodies as indicated.



Online Figure III. HUVECs were transfected with scramble or PKAC α siRNA (10 nM) against the α isofrom of the catalytic unit of PKA. Forty eight hours after transfection, the cells were subjected to laminar shear stress (12 dyne/cm²) for 5 or 15 min. Cells kept under static condition were used as control (time 0). Cell lysates were resolved by SDS-PAGE and blotted with various antibodies as indicated.

| Peptide | Sequence | Monoisotopic mass | | Charge state | m/z ^B |
|-----------------------|---|-------------------------------|-------------------------------|----------------|----------------------------|
| | | nonphosphorylated | phosphorylated | U | |
| SAMS S633 S1177 | HMRSAM <u>S</u> GLHLVKRR ^A PLVSSWRRKRKE <u>S</u> SNTDSA RTQEVTSRIRTQ <u>S</u> FSLQER | 1777.97 2203.15 2321.22 | 1857.93 2283.11 2401.19 | 4+ 4+ 3+ | 465.49 571.79 801.40 |

Online Table I. The sequence, mass, and m/z for the phosphorylation of SAMS, S633, and S1177 peptides

^A the putative Ser phosphorylation sites ^B m/z, 3+ (801.40) represents phosphorylated S1177 whereas m/z 4+ (465.49 and 571.79) are those for phosphorylated SAMS and S633.